

Supporting Information

Construction of the pSNAR-HA-LdNT2-RV vector.

For the construction of the HA-tagged *LdNT2* gene, the ATG start codon of *LdNT2* within a 5 kb EcoRV fragment in the pSNAR vector (Carter *et al.*, 2000) was mutagenized by the QuikChange site-directed mutagenesis method using the primer sequences, 5'-ACGAAGCTGAAGTTGGAA**TCCGGA**CGGGCCAATCTGCTGC-3' (sense strand) and 5'-GCAGCAGATTGGCCCG**TCCGGA**TTCCAACTTCAGCTTCGT-3' (antisense strand), to introduce a BspEI site (bold and underlined). A leishmanial codon-optimized HA tag was synthesized from the following two oligonucleotides, 5'-ccggAAGA ATG tac ccg tac gac gtg ccg gac tac gc (sense strand) and 5'-ccgg gc gta gtc cg^g cac gtc gta cg^g gta CAT TCTT (antisense strand), that when annealed together resulted in a four base overhang at either end. These four base overhangs were ligated into the BspEI site of the mutagenized *ldnt2* gene within the pSNAR vector, resulting in the insertion of the leishmanial codon-optimized HA tag upstream but inframe with the *LdNT2* coding sequence. Note that this strategy led to the regeneration of a BspEI site at the 5'-end of the inserted HA tag.

Table S1. List of oligonucleotide sequences used in the qRT-PCR analyses

Gene Name	Primer Sequences	Accession Number
Aldehyde reductase, putative	(F) 5'- AAGGTGCTGCCAACAAACG-3' (R) 5'-AGGCATCACAGCTGCGAAA-3'	AM502249/A4I792
<i>L.donovani</i> HGPRT	(F1) 5'-CGACCTTGCCCCGTTCT-3' (R1) 5'-ACGAGCTCGCGCAAATAAAC-3' (F2) 5'-AGTGCAGCAAAGAAGATTGCA-3' (R2) 5'-CAGCAGGTAGAGCGGGTTGT-3'	AF170105
<i>L.donovani</i> XPRT	(F) 5'-CGGCCAGATCTCGATGCT-3' (R) 5'-TGCTTGCCCGTAGATTCT-3'	AF170105
<i>L.donovani</i> APRT	(F) 5'-CTTGAGATCCCCTCGTAGCTGCTA-3' (R) 5'-GCGGATAAGGAGACCAGCATT-3'	L25411
LdNT1	(F) 5'-CCCGTCAATGCCGTCTTC-3' (R) 5'-GGCGTAGCGGTAGTACGTCATA-3'	AF065311
LdNT2	(F1) 5'-TGATGCGGATCGGATGGT-3' (R1) 5'-ATTGCTCCGTCGACGTTATG-3' (F2) 5'-TCGCCGAAGCTCGTGATT-3' (R2) 5'-GCACGATCAGCGGAATAACA-3' (F3) 5'-TCGTCACTGAGATCGCTTCACT-3' (R3) 5'-CGAATGCCAAGCGGAATC-3'	AF245276
LdNT3	(F) 5'-CCAGCGTCAACCCTTACCA-3' (R) 5'-GATGCCGTAGATCTCATTGC-3'	HM147244
LdNT4	(F) 5'-GCGGTTCCGGGTCTCTAGT-3' (R) 5'-TAGGTGTCGGACATGCTTACCTT-3'	HM147245
<i>L.donovani</i> adenine amino hydrolase	(F2) 5'-ACGGAGATGGCGTCAAC-3' (R2) 5'-GCCTTGAATCTGCCACAGT-3'	DQ093583
<i>L.donovani</i> 3'-nucleotidase	(F) 5'-CTTGAGATCCCCTCGTAGCTA-3' (R) 5'-GCGGATAAGGAGACCAGCATT-3'	L35078
<i>L.donovani</i> membrane acid phosphatase	(F) 5'-GTGCTATCGGCGAGTTGC-3' (R) 5'-AGGGCTCTCCACCAATGAGA-3'	AF149839
Nucleoside hydrolase-like protein	(F) 5'-AGGTGACGGTGGACTGCTCTA-3' (R) 5'-GGCGGCCTATCCATTCTG-3'	AM502232/LinJ14.0130
<i>L.infantum</i> PRPPS2	(F) 5'-TGTCTTCGGCGAAACGAATT-3'	LinJ33_V3.2040

cAMP specific phosphodiesterase, putative	(R)	5'-TGGCGGCTATA CGCGTAGT-3'	
	(F)	5'-AGAACGCCACCTCCATTGAT-3'	LinJ15_1540
	(R)	5'-CACCATAGCCTCTGCCTTCTG-3'	
Methylthioadenosine phosphorylase, (putative)	(F)	5'-CAACATCTGCGCGCTGAA-3'	LinJ05_V3.0830
Adenosine Kinase, putative	(R)	5'-AGCGATCCAACAGCGTTGA-3'	
	(F)	5'-AAGGAAGCCGCGGAGAAG-3'	LinJ30_V3.0940
	(R)	5'-GGTGGGAGCCTTGTGT-3'	
<i>L. infantum</i> OMPDC-OPRT	(F)	5'-CAGCCTTCAGCCGTTCATG-3'	LinJ16_V30560
	(R)	5'-CGACGTCTTGCAAAGTACAAACA-3'	
<i>L. donovani</i> ADSS	(F)	5'-GAAACGCAACGCCATAACG-3'	EF999428
	(R)	5'-TTCTGGCGCTATTGGTGT-3'	
<i>L. donovani</i> ASL	(F)	5'-TTCATCCACTTGGGCTAACGT-3'	EF999429
	(R)	5'-GCAGGAGCATCGGAATCG-3'	
<i>L. donovani</i> IMPDH	(F)	5'-TCGGTGCCTCCGAACACT-3'	M55667
	(R)	5'-GATGCCCTTTCCTCCTTGAT-3'	
<i>L. infantum</i> GMPS	(F)	5'-CGGGAGCAGATGGCTTGA-3'	LinJ22_V3.0013
	(R)	5'-GAGGCGCGTAATGCAGATG-3'	
<i>L. infantum</i> GMPR	(F1)	5'-CGGGAGCAGATGGCTTGA-3'	LinJ17_V3.0870
	(R1)	5'-GAGGCGCGTAATGCAGATG-3'	
Putative glycosomal AMPDA	(F)	5'-GTCATCATCGTCTGGCATTATG-3'	LinJ35_V3.4860
	(R)	5'-TCTCCGCATTAAGCAAAAAGAAC-3	

Table S2. Analysis of gene expression in cells starved of purine for 48 h.

Gene Name	ΔC_T		$\Delta\Delta C_T$	$2^{-\Delta C_T}$
	Replete	Starved		
APRT	-2.6 ± 0.06	-1.72 ± 0.01	-0.88	1.8
HGPRT _A EXPT1	-2.56 ± 0.01	-3.29 ± 0.10	-0.73	1.7
HGPRT _A EXPT2	-1.52 ± 0.17	-2.44 ± 0.06	-0.92	1.9
HGPRT _B	-1.50 ± 0.22	-2.46 ± 0.06	-0.96	1.9
XPRT	-1.42 ± 0.10	-3.06 ± 0.01	-1.6	3.1
LdNT1	-4.79 ± 0.13	-5.50 ± 0.11	-0.71	1.6
LdNT2 _A	-4.96 ± 0.09	-3.65 ± 0.05	1.31	0.40
LdNT3	-3.56 ± 0.10	-5.63 ± 0.06	-2.1	4.2
LdNT4	-1.92 ± 0.10	-2.68 ± 0.09	-0.76	1.7
3'-Nucleotidase	-1.66 ± 0.10	-2.48 ± 0.06	-0.82	1.8
Acid Phosphatase	-5.62 ± 0.09	-6.46 ± 0.06	0.84	1.8
MTAP	-0.10 ± 0.13	-1.19 ± 0.08	-1.09	2.1
Adenosine Kinase	-2.52 ± 0.22	-2.88 ± 0.09	-0.36	1.3
PRPPS2	0.34 ± 0.22	-0.33 ± 0.09	-0.67	1.6
Nucleoside H	-2.19 ± 0.92	-2.36 ± 0.11	-0.17	1.1
AMPDA	0.78 ± 0.13	0.29 ± 0.04	-0.49	1.4
IMPDH	-1.64 ± 0.12	-1.72 ± 0.06	-0.08	1.1
GMPS	-0.50 ± 0.16	-0.95 ± 0.07	-0.45	1.4
ADSS	-1.05 ± 0.25	-1.35 ± 0.17	-0.30	1.2
ADSL	0.80 ± 0.22	0.73 ± 0.21	-0.07	1.0
cAMP-PDase	-1.36 ± 0.19	-1.29 ± 0.12	0.07	1.0

Relative mRNA abundances for genes involved in purine metabolism were determined via qRT-PCR according to the relative abundance method of (Livak & Schmittgen, 2001). For each gene, the ΔC_T for starved and replete samples represents the mean threshold cycle from triplicate technical replicates normalized to the mean threshold cycle of the endogenous control gene aldehyde reductase. Standard deviation was determined according to the method described in Applied Biosystems User Bulletin No. 2 (P/N 4303859). $\Delta\Delta C_T$ was determined by subtracting the mean ΔC_T of the starved samples from the mean ΔC_T of the replete samples and the overall fold change was calculated from the formula $2^{-\Delta\Delta C_T}$. Primer sequences and gene accession numbers are listed in Table S1. Subscripts A and B indicate that the qRT-PCR was performed using primer sets F1/R1 and F2/R2 respectively, as described in Table S1. HGPRT_{A EXPT1} and HGPRT_{A EXPT2} reflect the results of two independent experiments using primer pair F1/R1. Abbreviations: MTAP = Methylthioadenosine phosphorylase; PRPPS2 = putative glycosomal 5-phosphoribosyl-1- pyrophosphate synthetase; Nucleoside H = 6-oxopurine nucleoside hydrolase; AMPDA = adenosine monophosphate deaminase; IMPDH = inosine monophosphate dehydrogenase; GMPS = guanosine monophosphate synthase; ADSS = adenylosuccinate synthetase; ADSL = adenylosuccinate lyase; cAMP-PDase = cyclic adenosine monophosphate phosphodiesterase.

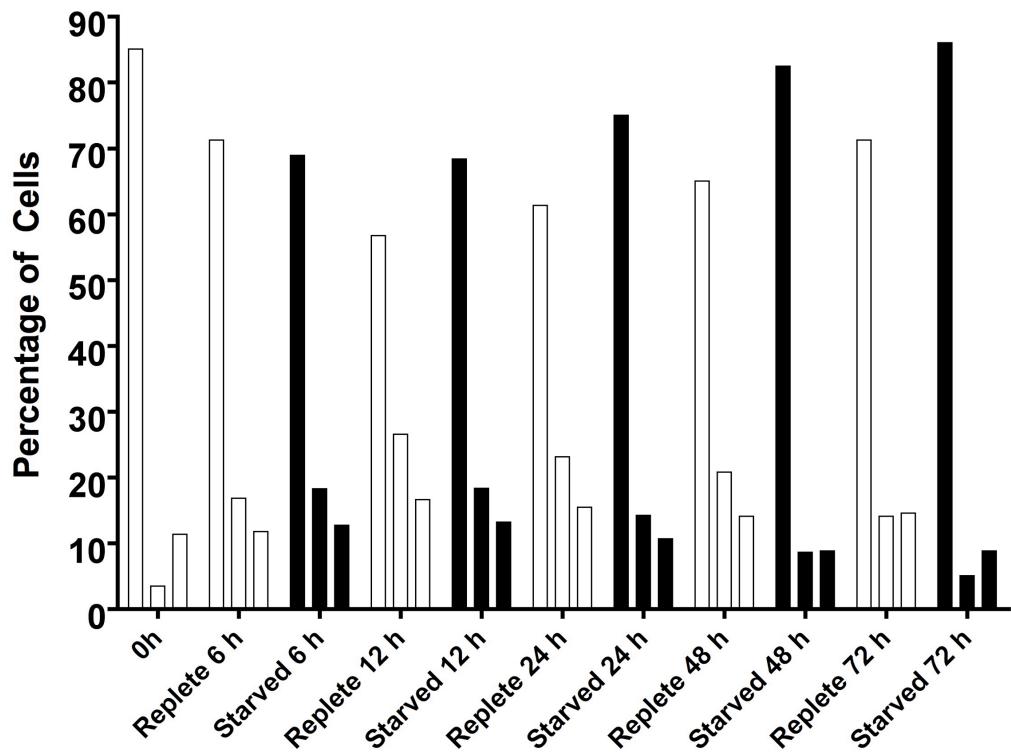


Fig. S1. The Effects of Purine Withdrawal on Cell Cycle Progression in *L. donovani*.

FACS analyses on fixed and propidium iodide stained purine-replete (open bars) and purine-starved (closed bars) LdB0b promastigote cultures were performed as described in *Experimental procedures*. Results are shown as percentage of cells in G1, S or G2 phase versus time in hours.